Magnetosensation in zebrafish

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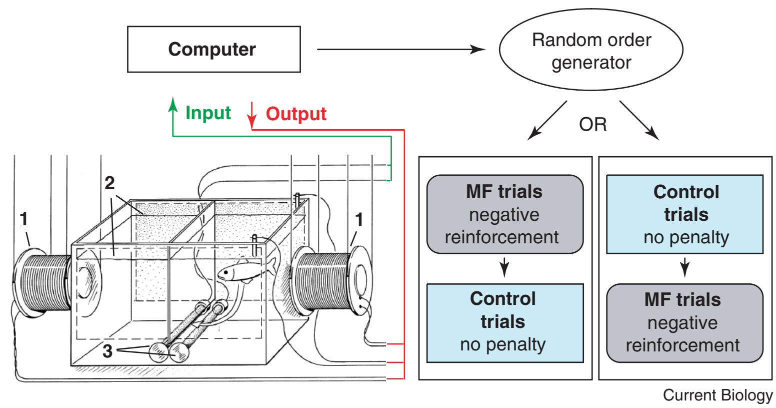

Figure 1. Testing magnetosensation in tilapia and zebrafish.

Fish were placed in an experimental module, consisting of two connected compartments. Magnetic field alterations were introduced by magnetic coils (1). Lack of escape upon magnetic field change triggered penalties – weak electrical impulses generated by non-magnetic metal plates (2) — at the end of the trial period. Escape into the other compartment was monitored by infrared light barriers (3) and disabled the punishment trigger. A computer-controlled random order generator produced trial pairs in random order, in which a magnetic field trial was followed by a control trial or vice versa.

Many species, from bacteria to vertebrates, have been reported to use the geomagnetic field as a major cue for oriented short and long range migration [1–10], but the molecular nature of the underlying receptor has remained elusive. One of the main reasons may be that past attempts to train animals to respond to magnetic stimuli proved surprisingly difficult [11]. We present a novel approach to magnetic conditioning, using a fast, fully automated assay system relying on negative reinforcement. Weak electric impulses were applied to punish fish that failed to escape upon magnetic field alterations (avoidance behaviour). Using this assay we first demonstrate magnetosensation in Mozambique tilapia, a fish migrating regularly between freshwater and the sea. Next we wondered whether non-migratory fish have a magnetic sense, such as zebrafish, the genetic fish model organism. Zebrafish were trained in groups of 4 individuals, and statistically highly significant reactions to magnetic field changes were recorded. The demonstration of magnetosensation in zebrafish opens a possibility to genetically identify the magnetoreceptor and its downstream signalling cascade.

Magnetoreception has previously been shown in migratory fish, such as tuna, salmon, and rainbow trout [4,11]. Before testing zebrafish, a non-migratory fish, we established a fully automated module using trout as test species. Following published procedures [4], a behavioural assay using positive reinforcement was developed. These experiments (Supplemental data), confirmed the presence of magnetosensation in trout [4]. Training single fish, however, took 30–40 days, rendering this assay inappropriate for a systematic genetic screening approach.

We therefore developed a second assay relying on negative reinforcement. The experimental set-up was composed of a fish tank with two conjoined compartments (Figure 1). A magnetic field (100μT) was applied in the east–west direction and subsequently fish that failed to escape to the respective other compartment were punished. The punishment was given as weak electric impulses of 3V. Successful escape was registered by an infrared light barrier system and disabled punishment. Trials were performed in pairs such that a magnetic field trial was always followed by a control trial or vice versa, in a randomly generated order (Figure 1). A total of 10 trial pairs per training session were performed, and fish were trained for a total of up to 10 sessions per day. Typically, training sessions were restricted to 3–4 on a single day. Data were sampled continuously and displayed as the ratio of infrared light barrier crossing (sensor signals) in magnetic field versus control trials, i.e., movement activity in each individual control trial was set to a value of one.

Mozambique tilapia, a robust migratory species, was chosen as a first experimental fish for avoidance training. Figure 2A summarizes the results on three fish trained individually in 10 sessions each. Learning effects were obvious from the fourth training session onward, with a total of 28% higher movement activity in magnetic field trials compared to control trials. The results were highly significant ($p<0.01$), and differences in response rates between magnetic field and control trials were within the same range as those obtained with trout in positive reinforcement conditioning (Supplemental data).

In a second set of negative conditioning experiments, we tested zebrafish for magnetosensation. As zebrafish are schooling fish, groups of four individuals were trained together. In order to establish experimental conditions for this much smaller species, we initially tested light as a strong conditioning factor. Avoidance behaviour was very pronounced and instantaneously obvious (Supplemental data). The average movement activity in a total of three experiments (12 fish) was 40% higher in light trials than in control trials ($p<0.001$). Next, fish were challenged with magnetic field trials (Supplemental data). A single experiment with a strong response rate (20% higher in magnetic field trials) is shown in Figure 2B. While response rates varied between individual experiments (Figure 2C), statistical

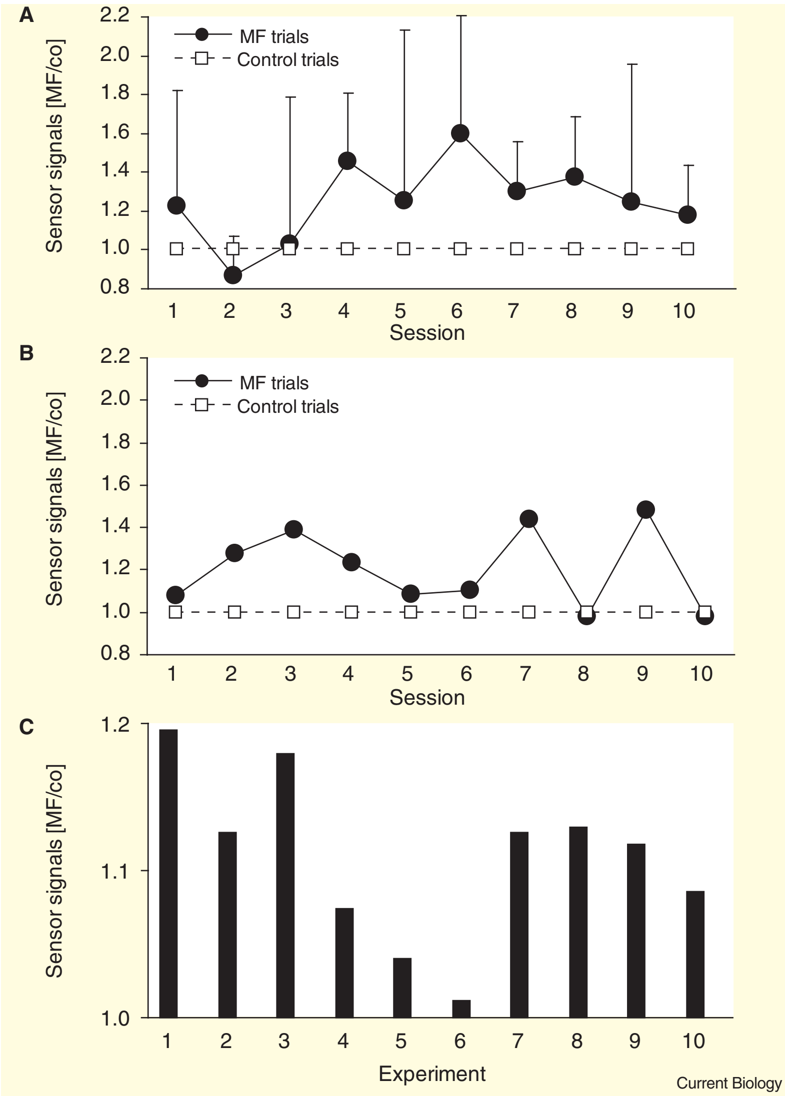

Figure 2. Magnetosensation in tilapia and zebrafish.

Results are expressed as relative response rates defined as the percentage of infrared sensor signals per magnetic field (MF) trial (or per control (co) trial) during a training session with 10 trial pairs. (A) Magnetosensation in tilapia. Mean response rates of three individual fish trained in 10 sessions of 10 trial pairs each. Bars indicate standard error. (B) Magnetic field conditioning experiment in zebrafish. Response rates of four fish trained as a group in 10 sessions of 10 trial pairs each. (C) Results from 10 group training experiments in zebrafish. Note that while response rates vary between experiments, they were higher in magnetic field trials than in control trials in all cases.

significance was high for the total of all 10 experiments (*p*<0.01).

Two sets of controls verified the presence of a magnetic sense in zebrafish. When infrared light sensor activations were counted during simulated, randomly generated trial pairs in which neither signal nor punishment was applied (software simulation), no differences were recorded (*p*=0.96). Another series of control experiments applied punishment in the absence of magnetic field changes. Again, no significant tendency was found (*p*=0.88).

Why should non-migratory fish have a magnetic sense? Short-range animals depend on dead reckoning, keeping track of outward legs while foraging and take the net displacement to plot a route home. Many species use the sun or polarized light for this task. In turbid water, under overcast skies and at night, however, the dead reckoning tool of choice may be the Earth's magnetic field. The demonstration of magnetosensation in zebrafish for the first time offers the opportunity to identify the magnetoreceptor and its downstream signalling cascade.

Supplemental data
Supplemental data containing experimental procedures are available at http://www.current-biology.com/cgi/content/full/15/5/R161/DC1/

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